FORM PTO-1390 (REV 12-29-99)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 2653/56

U.S. APPLICATION NO (If known, see 37 CFR 1.5): 10/070665

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US00/24597	September 8, 2000	September 8, 1999

TITLE OF INVENTION: POLYSIALIC ACID-KLH CONJUGATE VACCINE

APPLICANT(S) FOR DO/EO/US: LIVINGSTON, Philip O.; RAGUPATHI, Govindasnami

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☑ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☑ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☑ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☑ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☑ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☑ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☑ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☐ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☐ Other items or information:

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
10/070665	PCT/US00/24597	2653/56

17. ☑ The following fees are submitted:	CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$970.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$840.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$690.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$670.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$96.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 710.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED		NUMBER EXTRA	RATE		
Total claims	81	- 20 =	61	X **$18.00**	$ 1,098.00	
Independent claims	7	- 3 =	4	X **$78.00**	$ 336.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)				+ **$260.00**	$	
TOTAL OF ABOVE CALCULATIONS =					$ 2,144.00	
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also by filed (Note 37 CFR 1.9, 1.27, 1.28).					$ 1,072.00	
SUBTOTAL =					$1,072.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +					$	
TOTAL NATIONAL FEE =					$1,072.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +					$	
TOTAL FEES ENCLOSED =					$1,072.00	
					Amount to be refunded:	$
					charged:	$

a. ☐ A check in the amount of $_____ to cover the above fees is enclosed.

b. ☑ Please charge my Deposit Account No. 11-0600 in the amount of $1,072 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☑ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 11-0600. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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POLYSIALIC ACID-KLH CONJUGATE VACCINE

FIELD OF THE INVENTION

Polysialic acid is a tumor antigen found on the surface of small cell lung cancer (SCLC) and neuroblastoma cells. Normally, however, it is recognized as a self antigen by the immune system and ignored. In accordance with this invention, compositions of long chain polysialic acid polymers covalently conjugated to an immunogenic carrier and mixed with a saponin have been discovered which are effective immunogens to produce high titer antibodies useful for treating SCLC and neuroblastoma. In addition to the compositions, this invention provides active and passive immunization methods for treating SCLCs and neuroblastomas, as well as metastases of these cancers.

BACKGROUND OF THE INVENTION

In the United States, lung cancer remains the leading cause of cancer death in men, and has surpassed breast cancer as the leading cause of death in women. Small cell lung cancer (SCLC) accounts for approximately 20% of all lung cancer cases, and is the fifth leading cause of death from cancer (Wingo et al., 1995). Distant metastases are present in more than two-thirds of patients with SCLC at diagnosis, and in the absence of treatment, tumor progression is rapid, with a median survival of only 2 to 4 months. However, SCLC is responsive to chemotherapy, with over 80% of patients with limited stage disease (LD) and over 60% of patients with extensive stage disease (ED) achieving a major response to treatment. Despite these results, relapses are common, and most patients die within two years of their diagnosis. The median survival of patients with LD is 14-20 months, and those with ED is 8-12 months. Over the past decade, no additional therapy has been shown to improve overall survival, and standard therapy is observation alone for patients who have achieved a major response after 4 to 6 cycles of chemotherapy. Hence, new approaches to adjuvant therapy are needed.

Antibodies produced by B cells are the primary mechanism for the elimination of circulating pathogens from the bloodstream. They can cause rejection of allografts by both acute and chronic mechanisms. Antibodies induce destruction of cells by several mechanisms including opsonification and removal by the reticuloendothelial system, complement mediated lysis, and antibody-dependent cell mediated lysis. Thus, antibodies appear ideally suited for eradication of circulating tumor cells and micrometastases in the adjuvant setting (Livingston, 1995).

A study using monoclonal antibodies (mAbs) against polysialic acid demonstrated that this molecule is distributed on SCLC and neuroblastoma cells (Zhang, 1997). For example, polysialic acid was detected on 6 of 6 SCLCs and 5 of 5 neuroblastomas but not on any of the other cancer cells tested. Moreover, polysialic acid (as detected by mAbs 735 and NP-4) had a more restricted distribution on normal tissues than most of the other antigens, being detectable in grey matter of the brain by 735 but not NP-4, and in bronchial epithelia and some alveolar pneumocytes by both mAbs. NP-4 also showed some reactivity with secretory epithelia of stomach, colon and pancreas. While the possibility exists that immunization with polysialic acid could induce an autoimmune response against various subpopulations of cells, expression of polysialylated proteins on normal tissues seems to be minimal and in privileged sites. In fact, individuals having high titer antibodies against these polysialic acid antigens and some patients with paraproteins against polysialic acid have been identified, with no evidence of autoimmunity (Pon et al., 1997).

Polysialic acid chains associated with certain cancers have been characterized and generally contain 14 or more sialic acid units linked by alpha-2,8 linkages. These long polysialic acid chains are covalently attached to the extracellular regions of the neural cell adhesion molecule (N-CAM) by post-translational modification. The median number of sialic acid units in the polymers may be as high as 50. The embryonal form of N-CAM, modified with long sialic acid polymers, is found on the great majority of neuroblastomas and SCLC cells (Troy, 1992; Zhang et al., 1997). Long polysialic acid chains are also found in developing neural tissues of the fetus. Apart from mammalian cells, long polysialic acid chains are found to be associated

with the outer membrane of *Escherichia coli* K1 and *Neisseria meningitidis* Group B (Rougon et al., 1986). The difference between polysialic acid chains of bacteria and embryonal N-CAM relative to polysialic acid chains attached to N-CAM in mature tissue is the number of sialic acid units in the chain which may average 50 or more for the bacterial/embryonal form but fewer than 8 for the mature form. During post-natal maturation, the embryonal form is down-regulated (Friedlander et al., 1985), but can be found to be expressed on tumor cells (Roth et al., 1988; Komminoth et al., 1991) and certain types of normal cells (see, e.g., Husmann et al., 1989). For reasons that appear related to the secondary structure assumed by these longer polysialic acid chains, a series of monoclonal antibodies including mAbs 735 and NP-4 react strongly with embryonal neural tissue, neuroblastomas and SCLC tissues but only weakly or not at all with most normal tissues, which are known to express N-CAM with the shorter mature form polysialic acid (Hayrinen et al., 1995). Thus, the presence of antigenically distinct long polysialic acid chains on SCLC cells and neuroblastomas and the limited distribution of long polysialic acid chains in mature tissue suggests that it is an excellent target for immunotherapy.

The poor immunogenicity of polysialic acid is borne out by studies of high molecular weight polysaccharides extracted from group B strains of *N. meningitidis* (MenB) of which sialic acid was the major constituent (Gotschlich et al., 1969). In a study where antibody responses to group B polysaccharides were tested in human volunteers, out of 51 men tested, only one showed a confirmed rise in antibody (Wyle et al., 1972). On the basis of rabbit studies using MenB polysaccharide mixed with various adjuvants, only one of four volunteers immunized with MenB polysaccharide mixed with influenza vaccine developed a significant increase in antibody titer (Wyle et al., 1972). MenB polysaccharides coupled to tetanus toxoid failed to elicit polysaccharide specific antibodies in rabbits and mice (Jennings and Lugowski, 1981) and those antibodies actually produced were specific for the area of coupling between the MenB polysaccharide and the tetanus toxoid, rather than for the polysaccharide itself. To increase immunogenicity, *N. meningitidis* group B polysaccharides were modified by *N*-propionylation prior to immunization (Jennings et al., 1986).

However, not all of these antibodies raised against *N*-propionylated MenB polysaccharide were specific for native MenB polysaccharide (Hayrinen et al., 1995). Many antibodies reacted with proprionyl moiety. Accordingly, there remains a need for an immunogen and a method to induce an immune response against polysialic acid linked to N-CAM.

SUMMARY OF THE INVENTION

This invention is directed to an immunogenic composition comprising an α-(2→8)-polysialic acid-carrier conjugate and a saponin, wherein the α-(2→8)-polysialic acid-carrier conjugate comprises one or more α-(2→8)-polysialic acid polymers covalently linked to an immunogenic carrier, and wherein the median number of sialic acid units in each of the polymers is at least about 10. The polysialic acid polymers can also be modified to increase immunogenicity. Modifications include, but are not limited to, N-acylation and N-propionylation. Polysialic acid polymers have at least about 10 sialic acid units in order to stimulate immunity against polysialic acid polymers of the type found attached to embryonal N-CAM and can comprise as many as 200 or more sialic acid units. Polysialic acid polymers of the invention which are obtained from bacteria can average about 50 to 100 subunits or more and have a molecular weight of about 10,000 or more. Preferred immunogenic carriers include keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA). Preferred saponins are QS-21 and the semi-synthetic saponin GPI 0100.

Another aspect of the invention is directed to compositions useful as vaccines comprising amounts of the immunogenic compositions of the invention effective to induce antibodies specific for polysialic acid moieties of embryonal neural cell adhesion molecule (N-CAM) and a pharmaceutically-acceptable vehicle. The preferred adjuvant is a saponin and more preferably is QS-21 or GPI 0100.

The invention also provides a method for stimulating antibodies specific for polysialic acid of embryonal N-CAM by administering an amount of an immunogenic composition of the invention sufficient to stimulate antibodies specifically reactive

with embryonal N-CAM. Such antibodies are also reactive with SCLC or neuroblastoma cells having long chain polysialic acids.

Yet another aspect of the invention is directed to a method of treating SCLC or neuroblastoma in a subject or a patient by administering an amount of a composition comprising an α-(2→8)-polysialic acid-carrier conjugate and adjuvant in amount effective to ameliorate the cancer or neuroblastoma wherein the α-(2→8)-polysialic acid-carrier conjugate comprises one or more α-(2→8)-polysialic acid polymers covalently linked to an immunogenic carrier. This method is useful for further treatment of patients who have previously undergone primary treatment for SCLC or neuroblastoma (e.g., tumor removal and/or one or more rounds of chemotherapy). Similarly, this method can be used to treat metastases of SCLC or neuroblastoma. The preferred carrier used in this method is KLH and the preferred adjuvant is a saponin.

A still further aspect of this invention relates to a method of stimulating immunity against group B *N. meningitidis* or *E. coli* KI which comprises administering an amount of a composition comprising an α-(2→8)-polysialic acid-carrier conjugate and saponin in an amount effective to stimulate immunity against group B *N. meningitidis* or *E. coli* KI, wherein the α-(2→8)-polysialic acid-carrier conjugate comprises one or more α-(2→8)-polysialic acid polymers covalently linked to an immunogenic carrier, and wherein the median number or sialic acid units in each of the polymers is at least about 10.

The present invention further provides a method of passive immunization for treating SCLC or neuroblastoma in a subject by administering human or humanized antibodies specific for embryonal N-CAM to the subject in an amount effective to ameliorate the SCLC or neuroblastoma. Such antibodies can be administered prior to, concurrent with or after the subject has undergone a primary treatment for SCLC or neuroblastoma and is also useful for treating metastases of SCLC or neuroblastoma.

In yet another embodiment, the invention is directed to a method of passively immunizing a subject to treat or ameliorate infection caused by group B

N. meningitidis or *E. coli* K1 using antibodies raised in response to compositions of the invention.

DETAILED DESCRIPTION OF THE INVENTION

The compositions of the invention comprise polysialic acid conjugated to an immunogenic carrier and a saponin, the amounts of each being effective to stimulate or enhance antibody production in a human subject. These compositions can be formulated as vaccines by admixture with one or more pharmaceutically acceptable vehicles.

Polysialic acid polymers of the invention are substantially homopolymers of sialic acid of sufficient length to stimulate the production of antibodies which bind to long chain polysialic acid. Long chain polysialic acid consists of 10 or more sialic acid subunits. This is in contrast to polysialic acid present on normal cells which generally consist of 8 or fewer sialic acid units. It has been determined that the affinity of certain known monoclonal antibodies which bind to polysialic acid is significantly reduced when the polymer length is 8 sialic acid units or fewer. Such antibodies bind more efficiently when the polymer length is 10 or more. It has been suggested that binding of such antibodies requires that the polysialic acid polymer be in an extended conformation which is available only as the chain length increases beyond 8 sialic acid units. Preferred polysialic acid polymers contain 14 or more sialic acid units and can have up to about 200 sialic acid units. Polysialic acids obtained from bacteria typically can have a molecular weight of at least about 10,000 and can contain at least about 50 sialic acid units. Polysialic acid polymers can be modified to increase immunogenicity (e.g., N-propionylated α-(2→8)-polysialic acid).

In accordance with the invention, the amount of polysialic acid in an immunogenic composition used for immunization is between about 0.1 μg and about 1000 μg, and preferably is between about 1 μg and 100 μg and more preferably is about 30 μg. Hence, the amount of polysialic acid conjugate to administer depends on the total amount of sialic acid units present in the polysialic acid polymers conjugated to the carrier. The amount carrier can be determined by the conjugation

ratio of the polysialic acid to the carrier. For example, for conjugates having polysialic acid moieties linked to KLH, the molar ratio of polysialic acid to KLH ranges from about 50 to 1 to about 250 to 1. The molar ratio can be about 25 to 1 to about 1000 to 1. Where polysialic acid moieties are linked to small immunogenic carrier molecules, the molar ratio of polysialic acid to carrier can be less than 25.

As used herein, "immunogenic carrier" means a molecule which can initiate T lymphocyte activation that accompanies an antibody response. Immunogenic carriers have determinants that stimulate helper T cells to secrete cytokines which activate and stimulate proliferation of antibody-producing B cells. Hence, to stimulate or enhance an antibody response against an antigen of interest, particularly with poor immunogens, the antigen can be covalently linked (i.e., conjugated) to such immunogenic carriers. An immunogenic carrier of the invention can be a protein or portion of a protein which, when conjugated to polysialic acid, stimulates or enhances antibody production against the polysialic moieties in the subject. Examples of immunogenic carrier proteins are KLH and BSA. Immunogenic carriers also include polypeptides that are promiscuous class II activators (see, e.g., Panina-Bordignon et al., 1989) to which polysialic acid can be conjugated. In some cases, the number of polysialic acid polymers that can be conjugated to a polypeptide will not be large, but if the polypeptide carrier molecule is a particularly good promoter of a class II immune response, a sufficient immune response to polysialic acid can be obtained. In a preferred embodiment, the carrier is KLH or a derivative thereof. Conjugate linkages are made by well known methods.

In addition to immunogenic proteins and polypeptides which comprise a T cell epitope, carriers can also be constructs to which other immunogenic moieties (e.g., cytokines, polypeptides bearing T cell epitopes, etc.) can be linked. Branched constructs such as lipo-thioester and branched polylysine allow for multiple covalent linkages of such immunogenic moieties as well as conjugation of multiple polysialic acid polymers. Carriers further include proteins and polypeptides which have been modified by the covalent addition of immunogenically active moieties.

Polysialic acid-carrier conjugates are formulated with the adjuvant saponin to form the compositions of the invention. Saponins are immunological adjuvants that promote non-specific immune responses. The saponins in the compositions of the invention are present in an amount from about 1 μg to about 2000 μg. Saponins include semi-synthetic and synthetic saponins. In a preferred embodiment, the adjuvant is a saponin derived from the bark of a *Quillaja saponaria* Molina tree. A particularly preferred adjuvant is QS-21. QS-21 is preferably present in an amount between about 50 μg and about 500 μg or between about 50 μg and about 200 μg and more preferably in an amount of about 100 μg. Other saponins from *Quillaja saponaria* Molina may also be used, as well as saponins from other plant sources. Another example of a saponin source is *Saponaria officinalis*. The monosaccharide composition, molecular weight, adjuvant effect and toxicity for a series of saponins has been described (Kensil et al., 1991). A second particularly preferred adjuvant is the semi-synthetic saponin GPI 0100. GPI 0100 is preferably present in an amount between about 10 μg and about 2000 μg or between about 100 μg and about 2000 μg or between about 100 μg and about 1000 μg and more preferably in an amount of about 500 μg to about 1000 μg.

The ratio of polysialic acid and adjuvant used in compositions of the invention can vary. One of ordinary skill in the art can readily determine the effective amounts of conjugated polysialic acid to be used for immunization. Positive immune responses are indicated, for example, by an increased titer of antibodies specific for the embryonal form of N-CAM or by increased complement mediated lysis of cells having long chain polysialic acid on the cell surface (e.g. H345 cells) by immune sera relative to preimmune sera.

Vaccines of the invention comprise the compositions of the invention and a pharmaceutically acceptable vehicle. The vaccines can be administered intradermally, subcutaneously, intramuscularly or by any other suitable route for eliciting an immune response. For the purposes of the invention, "pharmaceutically acceptable vehicle" means any of the standard pharmaceutical vehicles. Examples of suitable vehicles are well known in the art and include, but are not limited to, any of the standard vehicles

such as phosphate buffered saline, phosphate buffered saline containing Polysorb, water, emulsions such as oil/water emulsion, and various types of wetting agents. In a preferred embodiment, vaccines of the invention are for administration to a human. Alternatively, a vaccine can be administered to any animal to induce the formation of antibodies which bind to a polysialic acid moiety of embryonal N-CAM or for producing humanized antibodies.

The invention further provides a method of stimulating the production of antibodies specific for polysialic acid moieties on N-CAM (or on bacteria carrying such moieties) in a subject which comprises administering a sufficient amount of a composition of the invention to stimulate antibody production. Stimulating antibody production means inducing the production of antibodies which bind to long-chain polysialic acid polymers in a subject wherein such antibodies were previously undetectable or detectable at only a low level or titer. Stimulation of antibody production as used herein includes producing increased amounts of polysialic acid-specific antibodies or antibodies with increased affinity for polysialic acid, or both.

Preferably, immunization with a composition of the invention is performed using multiple injections administered over a time course which is selected to maximize the titer and/or function of induced anti-polysialic acid antibodies. However, any suitable immunization regimen can be used. In a preferred embodiment, a composition of the invention for human administration comprises KLH, or a derivative thereof, conjugated to polysialic acid and mixed with the adjuvant saponin. The saponin is preferably QS-21.

Antibody titers in sera of immunized subjects can be determined by ELISA or by any other assay to measure antibodies as well known to one of skill in the art. One way to examine the function of such antibodies is to measure their ability to bind to polysialic acid on the surface of cells. For example, dilutions of antisera from immunized subjects can be added to cultured H345 cells, which express long chain polysialic acid polymers, and antibodies in the antisera detected with secondary antibodies which bind specifically to constant domains of the test antibodies. The secondary antibodies can be labeled, for example with an enzyme or a fluorescent tag.

A measure of the degree to which detectable label is associated with the H345 cells is a measure of the ability of induced antibodies to bind to polysialic acid on the surface of a cell. Another way to test antisera from immunized subjects is to add serial dilutions of antisera, together with human complement, to cultured H345 cells and observe killing of the H345 cells. Other ways to test for binding and function of anti-polysialic acid antibodies raised by immunization with compositions of the invention will be evident to those of ordinary skill in the art.

Another aspect of this invention provides a method of treating SCLC or neuroblastoma in a subject by administering to the subject an amount of a composition of the invention effective to ameliorate, reduce or otherwise treat the SCLC or neuroblastoma. In accordance with the invention, adjuvants other than saponin can be used in this method. For example, adjuvants which can be substituted for, or utilized in addition to, saponin include, but are not limited to, alum, oil-in-water emulsions, complete or incomplete Freund's Adjuvant, non-ionic block copolymers (e.g., TiterMax™, L121, CRL1005, etc.), adjuvant compositions which comprise bacteria derived substances such as monophospholipid A or cell wall skeleton (CWS), DNA having immunostimulatory CpG motifs and immunostimulatory cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin 2 (IL-2). Other common adjuvants well known to those of ordinary skill in the art can also be used.

Ameliorating SCLC or neuroblastoma means elimination or reduction of remnants of operable tumors which remain following surgical excision or other means of obliterating the tumor (e.g., a primary treatment). Moreover, the method is also useful for treating (e.g., eliminating or reducing) metastases and circulating cells that may give rise to metastases. For example, by the time SCLC is detected and treated, distant metastases are present in more than two-thirds of patients. While immunotherapy can be used as an adjuvant to chemotherapy, the present invention contemplates using immunotherapy as a substitute for chemotherapy, particularly for cancer patients who are not responsive to chemotherapy. The present method may also aid in preventing relapses in patients who have undergone successful primary

treatments. Hence, the present methods for treating SCLC or neuroblastoma are useful in a subject who has developed and been treated for SCLC or neuroblastoma and no longer displays any detectable signs of the cancer (i.e., a subject in remission).

Many ways are available to assess a patient's or subject's response to the immunotherapeutic methods of the invention. For example, methods of detecting cancerous cells or observing amelioration of the cancer are well known. An example of such a method is the detection of parathyroid hormone-related protein (PTHrP), which is expressed in a variety of human cancers including lung cancer, the COOH terminal of which is stable and measurable in urine (Nishigaki et al., 1999). Immunolabeling techniques are also available for detection of tumor cells in bone marrow of SCLC patients (Pelosi et al., 1999). Alternatively, reverse transcriptase polymerase chain reaction (RT-PCR) is increasingly used to detect small numbers of circulating tumor cells. Such cancer detection and surveillance techniques are applicable for both active and passive immunization methods.

Compositions of the invention can also be used to stimulate immunity against group B *N. meningitidis* or *E. coli* KI. As in methods described above, immunity can be shown by an increase in the titer and/or affinity of circulating antibodies capable of binding to the polysialic acid moieties on the embryonal form of N-CAM or to the polysialic acid moieties on the aforementioned bacteria. If necessary, increased titers of circulating antibodies can be maintained by a regular course of immunizations. However, following an initial course of vaccination, it is usually sufficient to administer booster vaccinations when needed to maintain the immune system in a state where it can respond to subsequent infection with a vigorous secondary immune response. The timing of administering booster vaccines can be determined by one of ordinary skill in the art.

A further aspect of the invention provides methods of passive immunization using antibodies obtained against the compositions of the invention. Such antibodies can be obtained, for example, by immunizing a subject with a composition of the invention and recovering sera from the subject. If desired the anti-polysialic acid antibodies can be further purified from the sera. It is well within the abilities of one

of average skill in the art to purify such antibodies from antisera of subjects that have been vaccinated with compositions of the invention, as well as to collect and immortalize B cells secreting anti-polysialic acid antibodies from such subjects. Antibodies useful for treatment of humans may be obtained from other sources as well. Methods for generating human and humanized antibodies are well known. For example, the binding site amino acid residues of a non-human antibody specific for polysialic acid which was obtained by immunizing an animal with a composition of the invention can be substituted into a human antibody to produce a chimeric antibody which is not foreign to the human immune system. Alternatively, transgenic animals which comprise human Ig genes can be vaccinated with compositions of the invention to obtain polyclonal antisera specific for polysialic acid, or for the production of hybridomas which secrete human monoclonal antibodies which are specific for polysialic acid. For a review of such methods, see, e.g., Vaughan et al. (1998).

Antibodies obtained by such methods can be used for treatment of SCLC and neuroblastoma immediately upon diagnosis and prior to the usual primary treatments. Such antibodies can also be administered to treat or prevent the formation of metastatic tumors concurrent with or following primary treatments.

The antibodies can also be administered in methods of passive immunization against group B *N. meningitidis* or *E. coli* K1. Such methods of treatment are especially useful for treating patients who may have acquired such an infection but whose immune systems have not had sufficient time to respond to and to eliminate the infection. Passive immunization can, for example, reduce the severity and duration of the infection. Methods and routes of administration are well known to those of ordinary skill in the art. Examples and reviews of such methods are readily available (see, e.g., Schirrmacher *et al.*, 1996; Saltzman, 1993; Masuho, 1992; Hammarstrom and Smith, 1990).

Throughout this application, various publications, patents, and patent applications have been referred to. The teachings and disclosures of these publications, patents, and patent applications in their entireties are hereby

incorporated by reference into this application to more fully describe the state of the art to which the present invention pertains.

It is to be understood and expected that variations in the principles of invention herein disclosed may be made by one skilled in the art and it is intended that such modifications are to be included within the scope of the present invention.

Examples of the invention which follow are set forth to further illustrate the invention and should not be construed to limit the invention in any way.

EXAMPLE 1

Antigen Preparation

Polysialic acid preparation - Poly-2,8-N-acetylneuraminic acid (colominic acid) is supplied as a powder (Sigma Chemical Co., St. Louis, MO; Nacalai Tesque, Inc., Kyoto, Japan) which contains a single major species of about 30,000 molecular weight (a homopolymer from *E. coli* of over 50 sialic acid units) and multiple species of lower molecular weight. Prior to conjugation to KLH, long polysialic acid chains were purified from lower molecular weight chains by gel filtration chromatography. The polysialic acid was purified using a Sephacryl S-200 column (flow rate 1 ml/min; column dimensions 90 cm x 2.5 cm) and eluted with sterile PBS by Fast Performance Liquid Chromatography (FPLC). The long chain fraction was used directly for conjugation to KLH.

Conjugation of polysialic acid to KLH - Conjugation of polysialic acid to KLH (Intracel Corp., Rockville, MD) was accomplished by the periodate method which involves the reaction of sodium metaperiodate with the vicinal hydroxyl groups in the polysialic acid chain to form a dialdehyde at the end of each chain. The dialdehyde is reacted with free amino groups of KLH in aqueous solution to yield diamine linkages which in turn are stabilized by reaction with sodium cyanoborohydride.

Preparation of polysialic acid dialdehyde - All glassware was rinsed with distilled water and autoclaved prior to use. 10 mg of purified polysialic acid was dissolved in 1 ml sodium phosphate buffer. 2.5 mg of sodium metaperiodate (freshly prepared) was added and the solution was stirred at room temperature (25°C) for 90 minutes. At the end of the incubation period, 2.9 mg (46.6 μmole) of ethylene glycol (2.6 μl; 1.11 mg/μl) may be added to neutralize unreacted sodium metaperiodate and the solution stirred for 15 min. at 25°C. Whether neutralized or not, excess sodium metaperiodate was removed by passage over a Sephadex G-10 column equilibrated with PBS buffer. Fractions were collected at one minute intervals and those fractions shown to be positive by reaction with 0.2% resorcinol were combined.

Conjugation of polysialic acid dialdehyde to KLH - The polysialic acid dialdehyde was transferred to a sterile glass bottle containing 5 mg of sterile, pyrogen-free KLH (Intracel Corp., Rockville, MD) dissolved in PBS (5 mg/ml). The flask was rinsed two times with 2.5 ml of PBS, and these washes were added to the KLH/polysialic acid dialdehyde mixture and allowed to incubate at 37°C for 60 minutes with gentle stirring. A 20 mg/ml solution of sodium cyanoborohydride ($NaBH_3CN$) in PBS was prepared and sterile filtered. 1 ml of the $NaBH_3CN$ solution was added to the KLH/polysialic acid dialdehyde mixture and incubated at 37°C for 48 hr.

Purification of polysialic acid-KLH glycoconjugate - The contents of the polysialic acid-KLH reaction vial were loaded on a Sephacryl S-200 column (flow rate 1 ml/min; column dimensions 90 cm x 2.5 cm) and eluted with sterile saline. The fractions positive for both KLH and polysialic acid were combined and sterile filtered with a 0.22 μm low protein-binding sterile, pyrogen-free filter and stored at -20°C. Protein and sialic acid content were determined by BioRad protein assay and resorcinol reaction, respectively. QS-21 was added to yield 100 μg/ml and the mixture was filtered through a 0.22 μm filter. Aliquots of 1 ml were added to sterile 2 ml vials (Nunc) and lyophilized, capped and stored at -30°C.

EXAMPLE 2

Immunization

CB6/F1 mice were immunized subcutaneously with KLH-conjugated polysialic acid and 10 μg of QS-21 adjuvant. The amount of polysialic acid administered, as determined by reaction with resorcinol, was 3 μg. The initial vaccination was followed by two identical booster vaccinations at one week intervals.

Blood was collected and the presence of antibodies capable of binding to sialic acid was determined in two ways. First, antibody titers were determined by ELISA for both IgG and IgM. Table 1 shows the titers of IgM and IgG in the serum of five mice immunized according to the above protocol and indicates the highest serum dilutions which yielded a signal in an ELISA assay using microtiter dishes coated with a polysialic acid-BSA conjugate. All 5 mice displayed significant increases in antibody titer for both IgM and combined IgG classes.

Table 1
Ab ELISA Titers Induced by Polysialic acid - KLH conjugate with QS-21

Mouse Number	Preimmune Serum		Immune Serum	
	IgM	IgG	IgM	IgG
1	0	0	200	6,400
2	0	0	1,600	6,400
3	0	0	1,600	25,600
4	0	0	200	12,800
5	0	0	1,600	12,800

The capacity of the serum antibodies to bind to surface antigen of H345 tumor cells (ATCC catalog no. HTB-180) was also tested. H345 cells were incubated with serum preparations from the five immunized mice and with preimmune serum. IgG and IgM antibodies were determined using fluorescein isothiocyanate (FITC)-conjugated secondary reagents specific for the selected antibody classes. Antibody bound to the H345 cells was determined by measuring fluorescence with a fluorescence-activated cell sorter (FACS). As shown in Table 2, the results of the FACS analysis demonstrate that the sera of all five mice produced antibodies capable

of binding to polysialic acid on tumor cells. Furthermore, the antisera promoted complement-induced tumor cell lysis *in vitro*.

Table 2
Antisera Reactivity with Tumor Cells by FACS Analysis

Mouse Number	% Positive IgM	% Positive IgG
1	36.44	81.63
2	90.02	93.37
3	91.72	89.41
4	93.83	88.78
5	92.37	94.96
Preimmune Serum	8.25	10.28

EXAMPLE 3

Immunization

Patients having completed initial therapy for SCLC and having achieved either a complete or partial response to therapy without subsequent disease progression were immunized subcutaneously with 30 μg of polysialic acid in polysialic acid-KLH conjugate and 100 μg of QS-21 adjuvant or 30 μg of N-proprionylated polysialic acid in N-proprionylated polysialic acid-KLH conjugate and 100 μg of QS-21 adjuvant. Four vaccinations were administered at one week intervals. A fifth vaccination was then administered following a four week interval (week 8) and a sixth vaccination was administered after a further eight week interval (week 16). Peripheral blood (20 - 30 ml) was drawn immediately before each vaccination and 2 weeks after the fourth, fifth and sixth vaccinations (weeks 6, 10 and 18).

Antibody responses were measured as described above in Example 2. As measured by ELISA assay using microtiter dishes coated with a polysialic acid-BSA conjugate and blocked with human serum albumin, four patients showed significant IgM responses to the N-propionylated polysialic acid-KLH conjugate administered with QS-21 (Table 3).

Table 3
Ab ELISA Titers against Polysialic acid - BSA conjugate Induced by Polysialic acid - KLH conjugate with QS-21 or N-propionylated Polysialic acid - KLH conjugate with QS-21

Vaccine	Patient	Median ELISA titer			
		IgM		IgG	
		Preimmune Serum	Immune Serum	Preimmune Serum	Immune Serum
PSA-KLH + QS-21	1	20	40	40	40
	2	10	10	20	20
	3	80	80	0	0
	4	0	20	0	0
	5	0	0	0	0
N-propionylated PSA-KLH + QS-21	1	10	320	0	0
	2	10	320	0	0
	3	40	1280	40	20
	4	10	20	0	0
	5	10	0	0	0
	6	0	80	0	40

H69 SCLC tumor cells (Carney et al., 1985; ATCC catalog no. HTB-180) were used to test the serum of immunized subjects for the presence of antibodies capable of binding to SCLC tumor cells (Table 4). Because preimmune sera usually shows binding to a variety of antigens present on the surface of the cells, a serum dilution was chosen for each subject which resulted in approximately 10% of H69 cells testing positive in FACS analysis with preimmune serum. This same dilution was applied to immune serum which was later obtained from that subject. The increases in % positive cells observed for immune sera as compared to preimmune sera should primarily reflect an increase in antibodies specific for polysialic acid displayed on H69 cells. In three subjects, the response to N-propionylated polysialic

acid-KLH conjugate plus QS-21 is detectable above the background level of serum antibodies which bind to other H69 antigens.

Table 4
Antisera Reactivity with Tumor Cells by FACS Analysis

Vaccine	Patient	Median Percent Positive Cells			
		IgM		IgG	
		Preimmune Serum	Immune Serum	Preimmune Serum	Immune Serum
PSA-KLH + QS-21	1	10	18	10	16
	2	10	8	11	12
	3	10	16	11	14
	4	10	6	11	15
	5	10	11	11	9
N-propionylated PSA-KLH + QS-21	1	11	48	11	14
	2	11	27	11	8
	3	10	15	10	24
	4	10	8	10	16
	5	11	10	11	5
	6	10	12	10	7

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WE CLAIM:

1. An immunogenic composition comprising an α-(2→8)-polysialic acid-carrier conjugate and a saponin, wherein the α-(2→8)-polysialic acid-carrier conjugate comprises one or more α-(2→8)-polysialic acid polymers covalently linked to an immunogenic carrier, wherein the median number of sialic acid units in each of the polymers is at least about 10.

2. The composition of Claim 1 wherein the α-(2→8)-polysialic acid polymer is modified to increase immunogenicity.

3. The composition of Claim 2 wherein modified α-(2→8)-polysialic acid polymer is N-propionylated α-(2→8)-polysialic acid.

4. The composition of Claim 1 wherein the α-(2→8)-polysialic acid-carrier conjugate consists essentially of one or more α-(2→8)-polysialic acid polymers covalently linked to an immunogenic carrier, wherein the median number or sialic acid units in each of the polymers is at least about 10.

5. The composition of Claim 1 wherein the median number of sialic acid units in each of the polymers is at least about 50, or wherein each of the polymers has an average molecular weight of at least about 10,000.

6. The composition of Claim 1 wherein the immunogenic carrier is keyhole limpet hemocyanin (KLH), an immunogenic derivative of KLH, bovine serum albumin (BSA), an immunogenic derivative of BSA, a promiscuous class II activating polypeptide, or an immunogenic derivative of a promiscuous class II activating polypeptide.

7. The composition of Claim 1 wherein the immunogenic carrier is KLH.

8. The composition of Claim 7 wherein the molar ratio of polysialic acid to KLH in the conjugate is from about 25 to about 1000.

9. The composition of Claim 7 wherein the molar ratio of polysialic acid to KLH in the conjugate is about 200.

10. The composition of Claim 1 wherein the amount of saponin is from about 1 μg to about 2000 μg.

11. The composition of Claim 1 wherein said saponin is QS-21 or GPI 0100.

12. The composition of Claim 11 wherein QS-21 is in an amount from about 50 μg to about 500 μg.

13. The composition of Claim 12 wherein the amount of QS-21 is about 100 μg.

14. The composition of Claim 11 wherein GPI 0100 is in an amount from about 100 μg to about 2000 μg.

15. The composition of Claim 14 wherein the amount of GPI 0100 is from about 500 μg about 1000 μg.

16. The composition of Claim 1 wherein the amount of the polysialic acid of said conjugate is from about 1 μg to about 1000 μg.

17. The composition of Claim 1, wherein the amount of the polysialic acid of said conjugate is about 30 μg.

18. The composition of Claim 1 wherein said composition is capable of inducing antibodies specific for polysialic acid moieties of embryonal neural cell adhesion molecule (N-CAM).

19. An immunogenic composition comprising an α-(2→8)-polysialic acid-KLH conjugate and QS-21 wherein the α-(2→8)-polysialic acid-carrier conjugate consists essentially of α-(2→8)-polysialic acid polymers covalently linked to KLH, and wherein the median number or sialic acid units in each of the polymers is 14 or greater.

20. A vaccine comprising an amount of the immunogenic composition of Claim 1 effective to induce antibodies specific for polysialic acid moieties of embryonal neural cell adhesion molecule (N-CAM) and a pharmaceutically acceptable vehicle.

21. A method for stimulating antibody production specific for polysialic acid of an embryonal N-CAM which comprises administering an amount of a composition comprising an α-(2→8)-polysialic acid-carrier conjugate and saponin effective to stimulate antibodies reactive with embryonal N-CAM, wherein the α-(2→8)-polysialic acid-carrier conjugate comprises one or more α-(2→8)-polysialic acid polymers covalently linked to an immunogenic carrier, and wherein the median number or sialic acid units in each of the polymers is at least about 10.

22. The method of Claim 21 wherein the α-(2→8)-polysialic acid polymer is modified to increase immunogenicity.

23. The method of Claim 22 wherein modified α-(2→8)-polysialic acid polymer is N-propionylated α-(2→8)-polysialic acid.

24. The method of Claim 21 wherein the α-(2→8)-polysialic acid-carrier conjugate consists essentially of one or more α-(2→8)-polysialic acid polymers covalently linked to an immunogenic carrier, wherein the median number or sialic acid units in each of the polymers is at least about 10.

25. The method of Claim 21 wherein the median number of sialic acid units in each of the polymers is at least about 50, or wherein each of the polymers has an average molecular weight of at least about 10,000.

26. The method of Claim 21 wherein the immunogenic carrier is keyhole limpet hemocyanin (KLH), an immunogenic derivative of KLH, bovine serum albumin (BSA), an immunogenic derivative of BSA, a promiscuous class II activating polypeptide, or an immunogenic derivative of a promiscuous class II activating polypeptide.

27. The method of Claim 21 wherein the immunogenic carrier is KLH.

28. The method of Claim 27 wherein the molar ratio of polysialic acid to KLH in the conjugate is from about 25 to about 1000.

29. The method of Claim 27 wherein the molar ratio of polysialic acid to KLH in the conjugate is about 200.

30. The method of Claim 21 wherein the amount of saponin is from about 1 μg to about 2000 μg.

31. The method of Claim 21 wherein said saponin is QS-21 or GPI 0100.

32. The method of Claim 31 wherein QS-21 is in an amount from about 50 μg to about 500 μg.

33. The method of Claim 32 wherein the amount of QS-21 is about 100 μg.

34. The method of Claim 31 wherein GPI 0100 is in an amount from about 100 μg to about 2000 μg.

35. The method of Claim 34 wherein the amount of GPI 0100 is from about 500 μg to about 1000 μg.

36. The method of Claim 21 wherein the amount of polysialic acid of said conjugate is from about 1 μg to about 1000 μg.

37. The method of Claim 21 wherein the amount of polysialic acid of said conjugate is about 30 μg.

38. The method of Claim 21 wherein said composition comprises an α-(2→8)-polysialic acid-KLH conjugate and QS-21 wherein the α-(2→8)-polysialic acid-carrier conjugate consists essentially of α-(2→8)-polysialic acid polymers covalently linked to KLH, and wherein the median number or sialic acid units in each of the polymers is 14 or greater.

39. A method of treating small cell lung cancer or neuroblastoma in a subject which comprises administering an amount of a composition comprising an α-(2→8)-polysialic acid-carrier conjugate and adjuvant in amount effective to ameliorate said small cell lung cancer or neuroblastoma, wherein the α-(2→8)-polysialic acid-carrier conjugate comprises one or more α-(2→8)-polysialic acid polymers covalently linked to an immunogenic carrier, and wherein the median number or sialic acid units in each of the polymers is at least about 10.

40. The method of Claim 39 wherein the α-(2→8)-polysialic acid polymer is modified to increase immunogenicity.

41. The method of Claim 40 wherein the modified α-(2→8)-polysialic acid polymer is N-propionylated α-(2→8)-polysialic acid.

42. The method of Claim 39 wherein the α-(2→8)-polysialic acid-carrier conjugate consists essentially of one or more α-(2→8)-polysialic acid polymers covalently linked to an immunogenic carrier, wherein the median number or sialic acid units in each of the polymers is at least about 10.

43. The method of Claim 39 wherein the median number of sialic acid units in each of the polymers is at least about 50, or wherein each of the polymers has an average molecular weight of at least about 10,000.

44. The method of Claim 39 wherein the immunogenic carrier is keyhole limpet hemocyanin (KLH), an immunogenic derivative of KLH, bovine serum albumin (BSA), an immunogenic derivative of BSA, a promiscuous class II activating polypeptide, or an immunogenic derivative of a promiscuous class II activating polypeptide.

45. The method of Claim 39 wherein the immunogenic carrier is KLH.

46. The method of Claim 45 wherein the molar ratio of polysialic acid to KLH in the conjugate is from about 25 to about 1000.

47. The method of Claim 45 wherein the molar ratio of polysialic acid to KLH in the conjugate is about 200.

48. The method of Claim 39 wherein said adjuvant comprises alum, a saponin, a semi-synthetic saponin-like molecule, CpG, GM-CSF, Freund's complete adjuvant, Freund's incomplete adjuvant or an oil-in-water emulsion.

49. The method of Claim 39 wherein said adjuvant is a saponin in an amount from about 1 μg to about 2000 μg.

50. The method of Claim 39 wherein said saponin is QS-21 or GPI 0100.

51. The method of Claim 50 wherein QS-21 is in an amount from about 50 μg to about 500 μg.

52. The method of Claim 51 wherein the amount of QS-21 is about 100 μg.

53. The method of Claim 50 wherein GPI 0100 is in an amount from about 100 μg to about 2000 μg.

54. The method of Claim 53 wherein the amount of GPI 0100 is from about 500 μg to about 1000 μg.

55. The method of Claim 39 wherein the amount of polysialic acid of said conjugate is from about 1 μg to about 1000 μg.

56. The method of Claim 39 wherein the amount of polysialic acid of said conjugate is about 30 μg.

57. The method of Claim 39 wherein said composition comprises an α-(2→8)-polysialic acid-KLH conjugate and QS-21 wherein the α-(2→8)-polysialic acid-carrier conjugate consists essentially of α-(2→8)-polysialic acid polymers

covalently linked to KLH, and wherein the median number or sialic acid units in each of the polymers is 14 or greater.

58. The method of Claim 39, wherein the composition is administered after the subject has undergone primary treatment for the small cell lung cancer or neuroblastoma.

59. The method of Claim 39, wherein the composition is administered to treat metastasis of said small cell lung cancer or neuroblastoma.

60. A method of stimulating immunity against group B *N. meningitidis* or *E. coli* KI which comprises administering an amount of a composition comprising an α-(2→8)-polysialic acid-carrier conjugate and saponin in an amount effective to stimulate immunity against group B *N. meningitidis* or *E. coli* KI, wherein the α-(2→8)-polysialic acid-carrier conjugate comprises one or more α-(2→8)-polysialic acid polymers covalently linked to an immunogenic carrier, and wherein the median number or sialic acid units in each of the polymers is at least about 10.

61. The method of Claim 60 wherein the α-(2→8)-polysialic acid polymer is modified to increase immunogenicity.

62. The method of Claim 61 wherein the modified α-(2→8)-polysialic acid polymer is N-propionylated α-(2→8)-polysialic acid.

63. The method of Claim 60 wherein the α-(2→8)-polysialic acid-carrier conjugate consists essentially of one or more α-(2→8)-polysialic acid polymers covalently linked to an immunogenic carrier, wherein the median number or sialic acid units in each of the polymers is at least about 10.

64. The method of Claim 60 wherein the median number of sialic acid units in each of the polymers is at least about 50, or wherein each of the polymers has an average molecular weight of at least about 10,000.

65. The method of Claim 60 wherein the immunogenic carrier is keyhole limpet hemocyanin (KLH), an immunogenic derivative of KLH, bovine serum albumin (BSA), an immunogenic derivative of BSA, a promiscuous class II activating polypeptide, or an immunogenic derivative of a promiscuous class II activating polypeptide.

66. The method of Claim 60 wherein the immunogenic carrier is KLH.

67. The method of Claim 66 wherein the molar ratio of polysialic acid to KLH in the conjugate is from about 25 to about 1000.

68. The method of Claim 66 wherein the molar ratio of polysialic acid to KLH in the conjugate is about 200.

69. The method of Claim 60 wherein the amount of saponin is from about 1 μg to about 2000 μg.

70. The method of Claim 60 wherein said saponin is QS-21 or GPI 0100.

71. The method of Claim 70 wherein QS-21 is in an amount from about 50 μg to about 500 μg.

72. The method of Claim 71 wherein the amount of QS-21 is about 100 μg.

73. The method of Claim 70 wherein GPI 0100 is in an amount from about 100 μg to about 2000 μg.

74. The method of Claim 73 wherein the amount of GPI 0100 is from about 500 μg to about 1000 μg.

75. The method of Claim 60 wherein the amount of polysialic acid of said conjugate is from about 1 μg to about 1000 μg.

76. The method of Claim 60 wherein the amount of polysialic acid of said conjugate is about 30 μg.

77. The method of Claim 60 wherein said composition comprises an α-(2→8)-polysialic acid-KLH conjugate and QS-21 wherein the α-(2→8)-polysialic acid-carrier conjugate consists essentially of α-(2→8)-polysialic acid polymers covalently linked to KLH, and wherein the median number or sialic acid units in each of the polymers is 14 or greater.

78. A method of treating small cell lung cancer (SCLC) or neuroblastoma in a subject which comprises administering human or humanized antibodies specific for embryonal N-CAM to the subject in an amount effective to ameliorate said small cell lung cancer or neuroblastoma.

79. The method of Claim 78 wherein the antibodies are administered prior to, concurrent with or after the subject has undergone primary treatment for said SCLC or neuroblastoma.

80. The method of Claim 78, wherein the antibodies are administered to treat metastasis of said SCLC or neuroblastoma.

81. A method of treating infection by group B *N. meningitidis* or *E. coli* K1 which comprises administering human or humanized antibodies specific for embryonal N-CAM to the subject in an amount effective to alleviate, reduce or eliminate said infection.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: POLYSIALIC ACID-KLH CONJUGATE VACCINE

(57) Abstract: The invention relates to compositions and vaccines comprising conjugates of polysialic acid which are capable of inducing antibodies which react effectively with polysialic acid on the surface of cancer cells and to methods of treatment which involve the stimulation or use of such antibodies.

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

DECLARATION AND POWER OF ATTORNEY	ATTY. DOCKET NO. 2653/56

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name,

I believe I am an original, first, and joint inventor of the subject matter that is claimed and for which a patent is sought on the invention entitled **POLYSIALIC ACID-KLH CONJUGATE VACCINE,** the specification of which was filed as International Application No. PCT/US00/24597 on the 8th day of September, 2000.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby claim the benefit under Title 35, United States Code § 120 of any United States Application or PCT International Application designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations § 1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. APPLICATIONS OR
PCT INTERNATIONAL APPLICATIONS
DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. § 120

U.S. APPLICATIONS
Number : 09/392,097
Filing Date : 08 September 1999

PCT APPLICATIONS
DESIGNATING THE U.S.
PCT Number : PCT/US00/24597
PCT Filing Date : 08 September 2000

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I declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issuing thereon.

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